

Colony PCR CYC_1 and self ligation

	PCR 20 µL reaction (units : µL)	Final Concentration			Primers:
10x thermopol reaction buffer	2,5	1X			f_IDT_squ_LS
10 mM dNTPs	0,5	200 µM			r_IDT_cir_LS
10 µM Forward Primer	0,5	0.2 µM			
10 µM Reverse Primer	0,5	0.2 µM			
Template (colony)	0	5 ng			DNA Template:
DMSO (optional)	0	3 %			Colonies
Taq polymerase	0,125	1.0 units/50 µl PCR			
Nuclease-free Water	20,875				
Total volume	25				
Number of reactions (including controls):	10	CYC_1 colonies 4-5-6 + 1			
WORK ON ICE					
Mastermix					
10x Thermopol reaction buffer	25				
10 mM dNTPs	5				
10 µM Forward primer	5				
10 µM Reverse primer	5				
DMSO (optional)	0				1 CYC_1_4-A
Phusion DNA Polymerase (ADDED LAST)	1,25				2 CYC_1_4-B
Nuclease-free Water	208,75				3 CYC_1_4-B bis
					4 CYC_1_5-B
Distribute 24 µl in tubes					5 CYC_1_6-A
					6 CYC1_6-B
					7 CYC self ligation A
					8 CYC self Ligation B
					9 CYC_1_5-A
					10 Negative control

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[illegible]